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# Using Leaf Ecological Stoichiometry to Direct the Management of *Ligularia virgaurea* on the Northeast Qinghai-Tibetan Plateau

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Leaf ecological stoichiometry not only reflects the plasticity and adaptability, but also the growth of plants within environments where temperature, precipitation, and soil properties vary across an elevation gradient. *Ligularia virgaurea* (Maxim.) Mattf. ex Rehder & Kobuski — an invasive poisonous plant — is common in the northeast portion of China's Qinghai-Tibetan Plateau and its presence greatly affects the native ecosystem. Based on *L. virgaurea* leaf carbon ($[C]_{leaf}$), nitrogen ($[N]_{leaf}$) and phosphorus ($[P]_{leaf}$) concentrations, and their ratios, the species' coping strategies across an elevation gradient (2,600 m, 3,000 m, and 3,300 m) were identified, and served to inform the development of improved management strategies. Mean $[C]_{leaf}$, $[N]_{leaf}$ and $[P]_{leaf}$ in *L. virgaurea* across all elevations were 413.14 $g{\cdot}kg^{-1}$, 22.76 $g{\cdot}kg^{-1}$, and 1.34 $g{\cdot}kg^{-1}$, respectively, while $[C]_{leaf}$: $[N]_{leaf}$, $[C]_{leaf}$: $[P]_{leaf}$, and $[N]_{leaf}$: $[P]_{leaf}$ were 18.27, 328.76, and 17.93. With an increase in precipitation and decrease in temperature from 2,600 m to 3,000 m–3,300 m, $[C]_{leaf}$, $[C]_{leaf}$: $[N]_{leaf}$ and $[C]_{leaf}$: $[P]_{leaf}$ of *L. virgaurea* decreased at first and then increased. The $[N]_{leaf}$ and $[P]_{leaf}$ gradually increased, whereas $[N]_{leaf}$: $[P]_{leaf}$ showed little change. Although temperature, precipitation and soil water content were the main factors affecting the ecological stoichiometry of *L. virgaurea* leaves, their roles in influencing leaf elements were different. The $[C]_{leaf}$ was mainly influenced by soil water content, $[N]_{leaf}$ by temperature and soil water content, and $[P]_{leaf}$ by all of them. With potential future climate change in the study area, *L. virgaurea* may grow faster than at present, although soil P may still be a growth-limiting element. As *L. virgaurea* can reduce plant diversity and the quality of forage, while increasing biomass, management of *L. virgaurea* should receive greater attention.

**Keywords: toxic weeds, alpine region, elevation, plant element concentration, warming**

## INTRODUCTION

Invasion by toxic weeds has become one of the critical causes of serious grassland degradation around the world (Goslee et al., 2001; Kleijn and Müller-Schärer, 2006; Timsina et al., 2011). Occupying over 60% of the total area of the Qinghai-Tibetan Plateau (QTP), alpine grasslands constitute a biome widely reported to be sensitive to human activities and climate change (Wu et al., 2012; Bakhshi et al., 2019). In recent years, in addition to *Ligularia virgaurea* (Maxim.) Mattf. ex Rehder & Kobuski, an increase in other poisonous and weedy plants (e.g., *Stellera chamaejasme* L., *Thermopsis lanceolata* R.Br., *Oxytropis ochrocephala* Bunge, *Gentiana straminea* Maxim., and *Potentilla bifurca* L.) has exacerbated grassland degradation on the QTP (Wu et al., 2012; Li et al., 2013; Xue et al., 2017). On the eastern QTP *L. virgaurea* is a poisonous and invasive grassland weed that is becoming increasingly common. It is also found in Bhutan and Nepal (Liu, 1989). As a strongly colonizing perennial herb bearing allelopathic toxins (Wang et al., 2018), *L. virgaurea* is an excellent indicator species of grassland degradation on the QTP (Wang et al., 2008). Its wide distribution has led to a reduction in grassland productivity, seriously threatening forage quality and sustainable development of animal husbandry in the region (Shi et al., 2018; Wang et al., 2018). While its chemical composition (Tori et al., 2006), seed germination and impact on soil biochemical factors (Shi et al., 2011; Shi et al., 2018) have been studied, its leaf ecological stoichiometry remains undocumented.

Carbon (C), nitrogen (N) and phosphorus (P) are plants' basic building blocks (Elser et al., 2000). Commonly known as ecological stoichiometry, the balance of multiple elements (mainly C, N and P) in plant tissues and its interaction with the ecosystem (Reich et al., 2006; Bradshaw et al., 2012; Jiang et al., 2019), provides a new theoretical approach to study the coupling of elements in the ecosystem, its structural and functional stability, and the means to its potential restoration (Güsewell and Bollens, 2003; Güsewell, 2010). Among the elements of interest, C is the most important in terms of dry matter and is the substrate and energy source essential to several physiological processes (Yang and Wang, 2011). In contrast, N and P, as key substrates in various biological processes operating in plants, are plants' main growth-limiting elements (Li et al., 2017; Müller et al., 2017).

In 1986, Reiners (1986) first explicitly incorporated stoichiometry as a complementary theory of ecosystem research by organically combining ecology with stoichiometry. Many studies on plants' ecological stoichiometry have followed. Plant ecological stoichiometry distribution patterns and their driving factors have been investigated at the regional scale (Sardans et al., 2011; Cao et al., 2020; Liu et al., 2021; Niu et al., 2021), and between different vegetation types (He et al., 2019; Qin et al., 2021). Differences in ecological stoichiometry have been noted in different plant organs (Zhang et al., 2017; Hu et al., 2018; Zhang et al., 2021), and under the influence of different disturbances: *e.g.*, shifts in soil moisture or nutrient content, invasive plant species, land degradation, and the implementation of various ecological recovery measures (Hu et al., 2018; Ouyang and Norton, 2019; Jing et al., 2020).

As plants' main photosynthetic organ, the leaf serves an important role in energy exchange and material transfer between the environment and plants (Sun et al., 2017b). Consequently, a leaf's stoichiometric features are closely related to the structure and function of the terrestrial ecosystem harboring the plant (Güsewell, 2010). The study of leaf ecological stoichiometry has become an effective method for determining plant growth rates, quantifying nutrient utilization efficiency and estimating the availability of soil nutrients for plant growth (Mcgroddy et al., 2004; Qin et al., 2021). Leaf ecological stoichiometry at the community or plant family level in terrestrial ecosystems has been intensively studied (Cao et al., 2020). On the northeast QTP, leaf ecological stoichiometry along an elevation gradient (Qin et al., 2021) has been explored at the community level, and for given individual plant species — e.g., *O. ochrocephala* (Cao et al., 2020), *S. chamaejasme* (Su et al., 2021), *P. bifurca* (Xu et al., 2018), *Leontopodium leontopodioides* (Xu et al., 2019). These studies have indicated that each species has its own specific leaf stoichiometry even when it faces the same environmental conditions.

The study of grassland degradation indicator species' ecological chemometrics and their driving factors can further elucidate grassland degradation mechanisms and inform the formulation of grassland management measures (Li et al., 2018). In the present case, investigation of leaf ecological stoichiometry of *L. virgaurea* at different elevations in the northeast QTP sought to elucidate the adaptive strategies of *L. virgaurea* to environmental changes through the regulation of its leaf ecological stoichiometry. Based on previous studies (e.g., Cao et al., 2020; Qin et al., 2021), our hypothesis was that leaf ecological stoichiometry of *L. virgaurea* would change with changes in temperature, precipitation, and soil physical and chemical properties linked to changes in elevation within the study area.

## MATERIALS AND METHODS

### The Study Area

Situated in the northeast QTP (lat. 38°32′–38°48′N, long. 100°03′–100°23′E), and ranging in elevation from 2000 to 4,700 m, the study area is subject to a typical continental climate. The region's minimum, maximum, and annual mean temperatures over 1987–2001 were -12.5°C, 19.6°C and 5.4°C, respectively (Yang et al., 2004). Over the same period, precipitation ranged between 300 and 500 mm $y^{-1}$, while evapotranspiration averaged 1,488 mm $y^{-1}$ (Yang et al., 2004). According to China's soil classification system, the main soil types were frigid calcic soils, aeolian soils, red earths, felty soils, paddy soils, castanozems and dark-brown earths, etc. In these mountainous areas, grasslands are predominantly situated on south-, east-, and west-facing slope aspects, while north-facing slopes are dominated by *Picea crassifolia* Kom. trees. The grassland vegetation includes *Carex tristachya* Thunb., *Kobresia bellardii* (All.) Degl. ex Loisel, *S. chamaejasme*, *Oxytropis alpina* Ten. and *L. virgaurea*.

## Experiment Design and Method

In August 2018, sampling sites were selected at three elevations: 2,600, 3,000, and 3,300 m amsl. At each site, three $10 \times 10\ m^2$ plots were randomly laid out, with 10 m between adjacent plots. Leaves of *L. virgaurea* were collected from five healthy plants with a similar degree of development within each plot. The leaves were taken from the middle of the plant, and stored in an envelope. After the leaves were sampled, three $1 \times 1\ m^2$ quadrats were set up along the diagonal (two ends and one midpoint) of each plot. At the center and four corners of each quadrat, soil samples were collected at three different depths (0–0.10 m, 0.10–0.20 m, and 0.20–0.40 m), using a 35 mm diameter drill. For each soil layer and each quadrat, the soil samples collected from the center and four corners were combined into a composite sample; this was then divided into two portions: one used to estimate soil moisture content, the other for the determination of soil physical and chemical properties.

After the fresh soil samples were weighed ($W_1$), they were dried in an oven at 105°C to a constant weight, $W_2$ (g). Gravimetric soil water content (SWC) was calculated as (Qin et al., 2019):

$$SWC = 100 \cdot \frac{W_1 - W_2}{W_2} \tag{1}$$

Leaves were dried (65°C, 48 h), ground, and their elemental (C, N, and P) concentrations determined. Samples for measuring soil physical and chemical properties were dried at room temperature in a darkened room. After impurities were removed, the soil was ground via a 2 mm mesh sieve, and the pH and soil nutrient levels were determined. A slurry of 1.0 g of dry soil and 2.5 ml of distilled water was placed in an Erlenmeyer flask and shaken for 10 min, then solids were allowed to settle 1–3 h. Soil pH was measured using a glass electrode connected to a pH meter PB-10 (Sartorius, Beijing, China) to measure the decanted supernatant. A volumetric potassium dichromate method was used to measure soil organic carbon (SOC) and leaf carbon concentration ($[C]_{leaf}$) (Nelson and Sommers, 1982). Following selenium-catalyzed $H_2SO_4$-$K_2SO_4$-$CuSO_4$ digestion of leaf or soil samples, soil total nitrogen (STN) and leaf nitrogen concentration ($[N]_{leaf}$) were determined (Bao, 2000). Using $H_2O_2$-$H_2SO_4$ digestion to extract P from leaf and soil material, soil total phosphorus (STP) and leaf phosphorus concentration ($[P]_{leaf}$) were determined colorimetrically (Olsen, 1954). Temperature and precipitation at the sampling sites were calculated according to temporal and spatial simulation empirical formulas (**Eqs. (2)** and **(3)** (Zhao et al., 2005; 2006):

$$\text{Temperature} = 20.96 - 5.49 \times 10^{-3}W - 0.17Q + 8.9 \times 10^{-3}Z, R^2 = 0.98 \tag{2}$$

$$\text{Precipitation} = 1.68 \times 10^3 + 0.12W + 12.41Q - 75.26Z, R^2 = 0.92 \tag{3}$$

where $W$ stands for elevation, $Q$ for latitude, $Z$ for longitude, and $R^2$ is the regression coefficient.

Differences in and fixed effects of elevation and random effect by plot were analysed using linear mixed model (details in section below) on soil physical and chemical properties, and on temperature and precipitation, among the sampling sites at different elevations, which are presented in **Table 1**.

## Data Analysis

For checking samples independence, linear mixed model (LMM) was used to determine the magnitude of elevation (fixed effects) and plot (random effects) on soil physical and chemical properties and leaf ecological stoichiometry of *L. virgaurea* (Liu et al., 2018). Differences in leaf ecological stoichiometry and soil properties of L. virgaurea at different elevations were examined using the *t*-test. Redundancy Analysis (RDA) was used to test the relationship between temperature, precipitation and soil physical and chemical properties and leaf ecological stoichiometry of *L. virgaurea* across elevations. After RDA analysis, the structural equation model (SEM) was used to further explore the indirect or direct effects of SWC, temperature, and precipitation on $[C]_{leaf}$, $[N]_{leaf}$ and $[P]_{leaf}$ (Wang et al., 2016). An insignificant $\chi^2$ test ($p > 0.05$), goodness fit index (GFI) (>0.90) and root mean square residual (RMR) (<0.05) were used to determine the adequacy of the SEM model fit (Hooper et al., 2008). Descriptive statistics and *t*-test were conducted in SPSS 22.0 (SPSS Inc. Chicago, United States). The LMM and RDA were performed by RStudio 4.0.1, while SEM was done using Amos 22.0 (Smallwaters Corporation, Chicago, IL, United States). The chosen significance level was $p \leq 0.05$.

# RESULTS

## The Independence of Leaf Samples Testing

The LMM showed that the effect of plot (random effects) on leaf ecological stoichiometry of *L. virgaurea* was not significant, while except for $[P]_{leaf}$ and $[N]_{leaf}$: $[P]_{leaf}$, elevation (fixed effects) exerted a significant effect on leaf ecological stoichiometry of *L. virgaurea* (**Table 2**).

## Variation in Leaf Ecological Stoichiometry of *L. virgaurea* at Different Elevations

From 2,600 m to 3,000 m–3,300 m, $[C]_{leaf}$, and $[C]_{leaf}$: $[N]_{leaf}$ first decreased and then increased, both showing a maximum value at 2,600 m (446.22 $g \cdot kg^{-1}$ and 21.36, respectively) (**Figures 1**, **2A,D**). The $[N]_{leaf}$ increased significantly with elevation, but $[P]_{leaf}$ increased more gradually, only the 2,600 m and 3,300 m elevations showing a significant difference (**Figures 2B,C**). Ranging from 178.71 to 617.55, $[C]_{leaf}$: $[P]_{leaf}$ at 2,600 m was

**TABLE 1 |** Soil properties, temperature and precipitation across the elevation gradient within the study area.

| Environmental factors | Elevation | | | Elevation (fixed effects) | | Plot (random effects) | |
|---|---|---|---|---|---|---|---|
| | 2600 m | 3000 m | 3300 m | $R^2$m | *p*-value | $R^2$c | *p*-value |
| SWC (%) | 6.61 ± 1.62 b | 5.64 ± 0.85 b | 21.09 ± 6.36 a | 0.7546 | 0.0024 | 0.8860 | 0.0144 |
| pH | 8.31 ± 0.09 b | 8.45 ± 0.08 a | 8.01 ± 0.12 c | 0.7926 | 0.0002 | 0.7926 | 0.9999 |
| SOC ($g \cdot kg^{-1}$) | 16.01 ± 2.84 b | 13.70 ± 2.07 b | 39.44 ± 10.19 a | 0.7696 | 0.0010 | 0.8415 | 0.1710 |
| STN ($g \cdot kg^{-1}$) | 1.06 ± 0.19 c | 1.50 ± 0.27 b | 3.81 ± 0.69 a | 0.8793 | 0.0001 | 0.9044 | 0.3619 |
| STP ($g \cdot kg^{-1}$) | 0.56 ± 0.10 a | 0.34 ± 0.10 b | 0.55 ± 0.11 a | 0.4803 | 0.0228 | 0.6322 | 0.2002 |
| SOC: STN | 15.18 ± 2.10 a | 9.20 ± 1.12 b | 10.23 ± 1.23 b | 0.7372 | 0.0011 | 0.7915 | 0.3653 |
| SOC: STP | 30.20 ± 10.57 c | 44.34 ± 16.59 b | 71.98 ± 11.03 a | 0.6424 | 0.0024 | 0.6794 | 0.6480 |
| STN: STP | 2.01 ± 0.75 c | 4.77 ± 1.53 b | 7.06 ± 1.01 a | 0.7729 | 0.0003 | 0.7729 | 0.9999 |
| LONG | 100°17′18″E | 100°14′26″E | 100°22′35″ E | — | — | — | — |
| LAT | 38°34′2.8″N | 38°33′22″N | 38°38′15″ N | — | — | — | — |
| T (°C) | 1.17 | −1.02 | −2.13 | — | — | — | — |
| P ($mm \cdot y^{-1}$) | 331.36 | 379.22 | 398.58 | — | — | — | — |

*Row-wise non-matching letters indicate a significant difference among elevations at $p \le 0.05$ level. pH: soil pH; SWC: soil water content; SOC: soil organic carbon concentration; STN: soil total nitrogen concentration; STP: soil total phosphorus concentration; LONG: longitude; LAT: latitude; $R^2$m: the degree of variation of the variable interpreted from the fixed effects; $R^2$c: the degree of variation of the variable interpreted combination from the fixed and random effects; T: temperature; P: precipitation; "—": Test was not done.*

**TABLE 2 |** Linear mixed model results of the effects of elevation and plot on leaf ecological stoichiometry of *L. virgaurea*.

| Variable | Elevation (fixed effects) | | | | Plot (random effects) | |
|---|---|---|---|---|---|---|
| | F | df | $R^2$m | p-value | $R^2$c | p-value |
| $[C]_{leaf}$ ($g \cdot kg^{-1}$) | 198.12 | 2 | 0.9384 | <0.0001 | 0.9384 | 1.0000 |
| $[N]_{leaf}$ ($g \cdot kg^{-1}$) | 197.09 | 2 | 0.9663 | <0.0001 | 0.9814 | 0.0460 |
| $[P]_{leaf}$ ($g \cdot kg^{-1}$) | 2.84 | 2 | 0.1790 | 0.1359 | 0.1790 | 0.9999 |
| $[C]_{leaf}$: $[N]_{leaf}$ | 319.24 | 2 | 0.9609 | <0.0001 | 0.9609 | 1.0000 |
| $[C]_{leaf}$: $[P]_{leaf}$ | 6.39 | 2 | 0.3295 | 0.0326 | 0.3295 | 0.9999 |
| $[N]_{leaf}$: $[P]_{leaf}$ | 0.91 | 2 | 0.0656 | 0.4506 | 0.0656 | 0.9999 |

*pH: soil pH; SWC: soil water content; SOC: soil organic carbon concentration; STN: soil total nitrogen concentration; STP: soil total phosphorus concentration; $[C]_{leaf}$: leaf carbon concentration; $[N]_{leaf}$: leaf nitrogen concentration; $[P]_{leaf}$: leaf phosphorus concentration. $R^2$m: the degree of variation of the variable interpreted from the fixed effects; $R^2$c: the degree of variation of the variable interpreted combination from the fixed and random effects.*

significantly greater than at other elevations, but no difference existed between the two higher elevations (**Figure 2E**); meanwhile $[N]_{leaf}$: $[P]_{leaf}$ showed no significant change across elevations (**Figure 2F**).

## Influence of Environmental Factors on the Ecological Stoichiometry of *L. virgaurea*

The RDA showed the first and second axes to collectively explain 59.11% of the total variation in *L. virgaurea* leaf ecological stoichiometry, with the first and second axes explaining 35.34 and 23.77% of the variation, respectively (**Figure 3**). Among the environmental factors, temperature, precipitation and SWC had the greatest effect on *L. virgaurea* leaf ecological stoichiometry (**Table 3**).

The results of the SEM showed that environmental factors can explain about 80, 94 and 21% of the variation of $[C]_{leaf}$, $[N]_{leaf}$ and $[P]_{leaf}$, respectively (**Figure 4**). The SWC indirectly affected $[C]_{leaf}$ through three paths: SOC: STN path, SOC-STP path, and SOC-pH path (**Figure 4A**). Temperature directly affected $[N]_{leaf}$, but SWC indirectly affected it through one path: SOC-pH (**Figure 4B**). Precipitation indirectly affected $[P]_{leaf}$ through three paths: STP-STN: STP path, SWC-STN: STP path and SWC-STP-STN: STP path, while temperature indirectly affected it through one path: STN: STP path (**Figure 4C**).

# DISCUSSION

## Dominant Factors Influencing the Ecological Stoichiometry of *L. virgaurea* Across Elevations

The results from the LMM showed that except for $[P]_{leaf}$ and $[N]_{leaf}$: $[P]_{leaf}$, leaf ecological stoichiometry of *L. virgaurea* was mainly determined by elevation, and not plot, suggesting the independence of samples (Liu et al., 2018). Change in environment as elevation increased from 2,600 to 3,300 m altered *L. virgaurea* leaf ecological stoichiometry to some extent, with the exception of $[N]_{leaf}$: $[P]_{leaf}$ (**Figure 2**), as was found in other studies (Liu et al., 2021; Su et al., 2021). However, sometimes, leaf ecological stoichiometry is related to the plant's inherited characteristics. For example, the leaves of long-lived and slow-growing plants often have a lower element concentration than ones from plants with contrasting features (Sun et al., 2017a).

In the present study, elevation mainly affected leaf ecological stoichiometry of *L. virgaurea* by influencing temperature, precipitation and SWC (**Table 3**), which is similar to the factors of influence reported by Su et al. (2021). This largely supported our hypothesis that leaf ecological stoichiometry of *L. virgaurea* would change with temperature and precipitation when elevation changes. Except for SWC, other soil physical and chemical properties had little direct effect on leaf ecological stoichiometry of *L. virgaurea*, only weakly supporting our

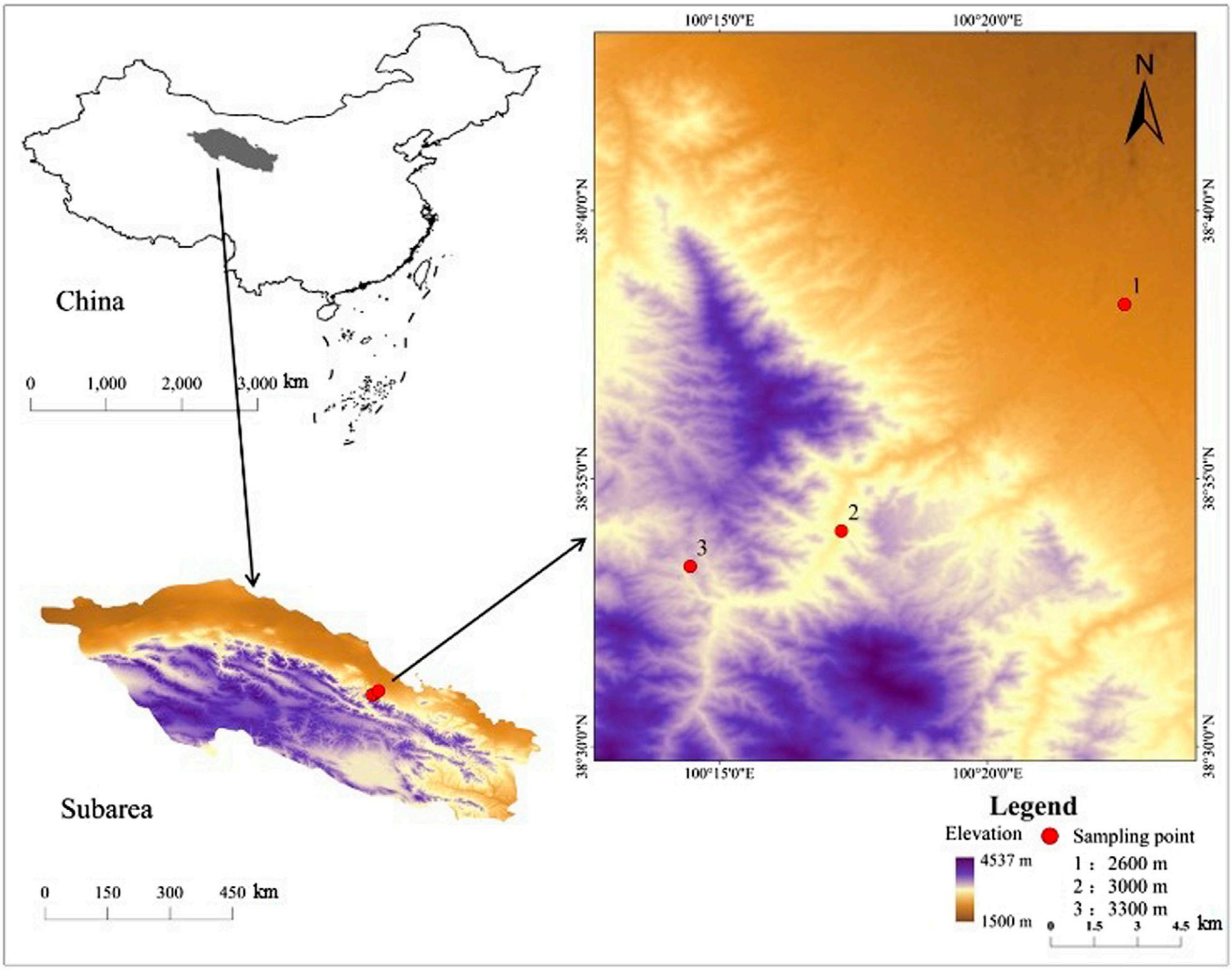


**FIGURE 1 |** Location of the sampling sites at 2,600, 3,000 and 3,300 m within the study area.

hypothesis that leaf ecological stoichiometry of *L. virgaurea* would change with soil physical and chemical properties when elevation changes. However, Liu et al. (2021) found that, under changes in elevation, the effects of temperature and precipitation on the leaf ecological stoichiometry of *Potentilla fruticosa* were not as great as pH, STP and SOC. This suggests that although elevation affects leaf ecological stoichiometry of different species, the specific influencing factors can be different, as the niche for each species differs (Silvertown, 2004). It is, therefore, that in understanding the adaptation strategies of a given species with changes of elevation, its leaf ecological stoichiometry must be independently studied.

## Mechanisms for the Dominant Factors Affecting $[C]_{leaf}$ Across Elevations

In general, in an environment with lower soil water content, leaves need to maintain a greater $[C]_{leaf}$ and specific leaf weight, as they must reduce photosynthetic and growth rates in order to reduce water consumption and improve their defenses against an adverse environment (Poorter and Bongers, 2006). Most studies have found that soil water content is negatively correlated with $[C]_{leaf}$ (Hu et al., 2018; Liu et al., 2020). In the present study, among these above-mentioned dominant factors, only SWC indirectly affected $[C]_{leaf}$ of *L. virgaurea* through three paths, namely SOC: STN path, SOC-STP path, and SOC-pH path (**Figure 4A**). The first path can be explained: as an important physical property of soil, soil moisture often has an important impact on other physical and chemical properties (Lin et al., 2021). For instance, with the increase of SWC, soil oxygen concentration and microbial activity will decrease (Borken and Matzner, 2009), which, in turn, will decrease the mineralization rate, as reflected by SOC: STN (Swift et al., 1979; Sanaullah et al., 2012). The other two paths can also be explained: an increase in SWC can lead to the cracking and dispersal of soil aggregates, which in turn dissolve and release forms of organic matter that are difficult for microorganisms to use, thus increasing SOC (Liu et al., 2014; Lin et al., 2021). With a change in SOC, pH and STP

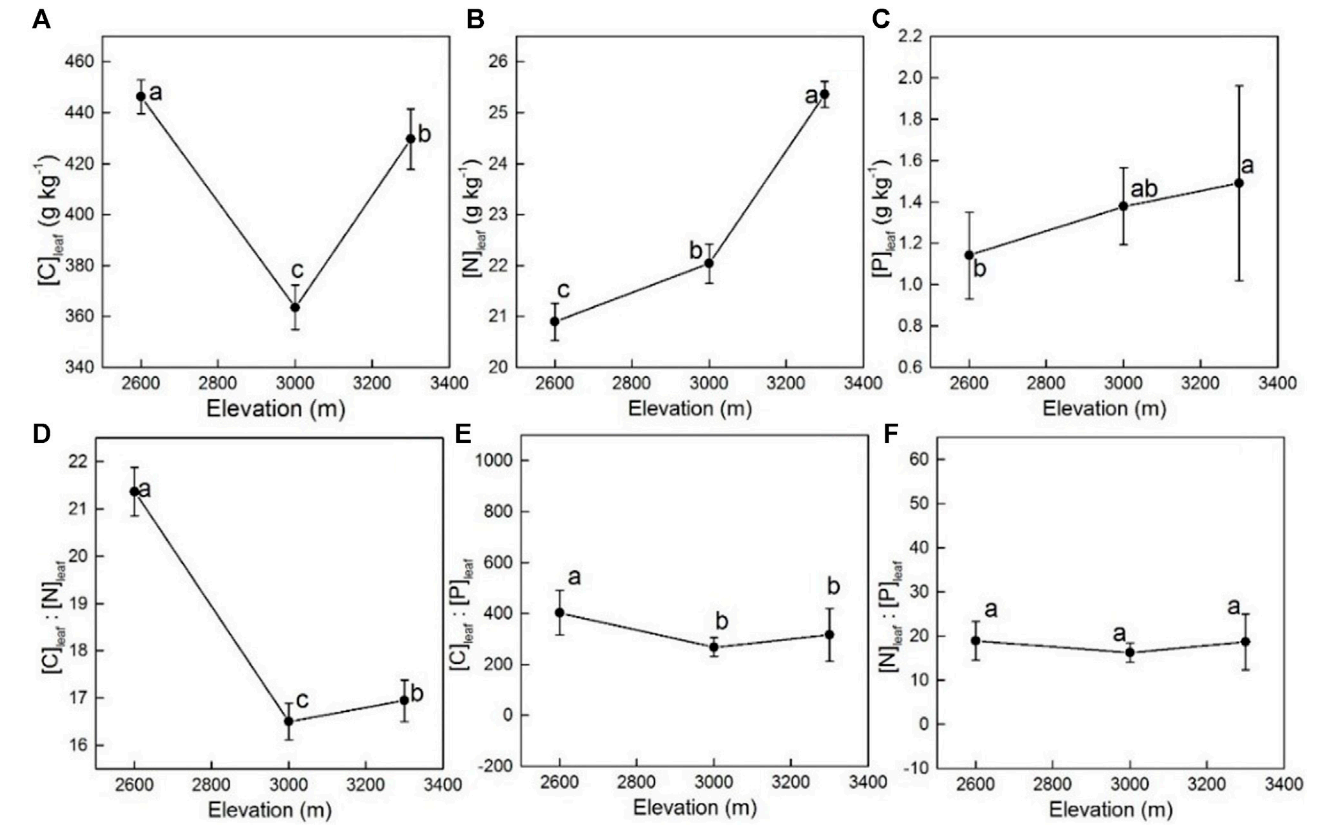


**FIGURE 2 |** *L. virgaurea* leaf carbon concentration **(A)**, leaf nitrogen concentration **(B)**, and leaf phosphorus concentration **(C)** and $[C]_{leaf}$: $[N]_{leaf}$ **(D)**, $[C]_{leaf}$: $[P]_{leaf}$ **(E)**, and $[N]_{leaf}$: $[P]_{leaf}$ **(F)** at different elevations. $[C]_{leaf}$, leaf carbon concentration; $[N]_{leaf}$, leaf nitrogen concentration; $[P]_{leaf}$, leaf phosphorus concentration.

will also be affected (**Figure 4A**). Soil acidity has been found to increase with increasing SOC due to the release of $H^+$ (Liu et al., 2019a). The relationship between SOC and STP is consistent with the findings of Cao et al. (2013) who found that increased soil organic matter can increase the STP, but the reason behind this requires further study.

With SOC: STN, STP and pH, as influenced by SWC, these exerted direct effects on $[C]_{leaf}$ of *L. virgaurea*. The average SOC: STN (11.54) was less than 25 in the study area, suggesting that the effective N produced by mineralization of organic matter could be used for plant growth (Haynes, 1986; Prescott et al., 2000; Li et al., 2019). With plant growth and photosynthesis enhanced (Tjoelker et al., 2010), $[C]_{leaf}$ would accumulate (**Figure 4A**). The STP is largely dependent on the soil parent body (Liu et al., 2005), and the P in the leaf is transported from the plant roots which take up P from the host soil (Parton et al., 1988). As the leaf's P increased with STP (**Figure 4A**), so $[C]_{leaf}$ increased, because P also participates in photosynthesis. The effect of pH on $[C]_{leaf}$ is opposite of that of SOC: STN and STP on $[C]_{leaf}$ (**Figure 4A**). It was found that higher pH values were often associated with a higher salt content (Kissel et al., 2009). The soil pH at different elevations in the study area was above 8 (**Table 1**), indicating a high salt content that would likely reduce stomatal conductivity (He et al., 2016) and water level in the leaf (Flowers and Colmer, 2008), thereby inhibiting leaf photosynthesis and reducing $[C]_{leaf}$.

## Mechanisms for the Dominant Factors Affecting $[N]_{leaf}$ Across Elevations

Temperature regulates the function of plant cells by affecting a series of physical and chemical reactions (*e.g.*, enzyme activity and membrane systems) that affect leaf ecological stoichiometry (Hall et al., 2010; Liu et al., 2019b). Temperature directly affected $[N]_{leaf}$ of *L. virgaurea*, while SWC indirectly affected it (**Figure 4B**).

The relationship between temperature and $[N]_{leaf}$ was consistent with that in most studies (Han et al., 2005; Chen et al., 2013) and the temperature-plant physiology hypothesis (Reich et al., 2004). This hypothesis posits that the decrease of temperature would result in a greater leaf nutrient concentration to compensate for the decline in metabolic rate, thereby improving plant tissues' adaptation to temperature and nutrition utilization. It was reflected by a significant increase in $[N]_{leaf}$ with the increase in elevation (**Figure 2B**). With pH influenced by SOC and SWC, it exerted a directly effect on $[N]_{leaf}$ of *L. virgaurea* (**Figure 4B**). The relationship between pH and $[N]_{leaf}$ was consistent with the results of Liu et al. (2021). As soil alkalinity decreased and acidity increased, the activity of some

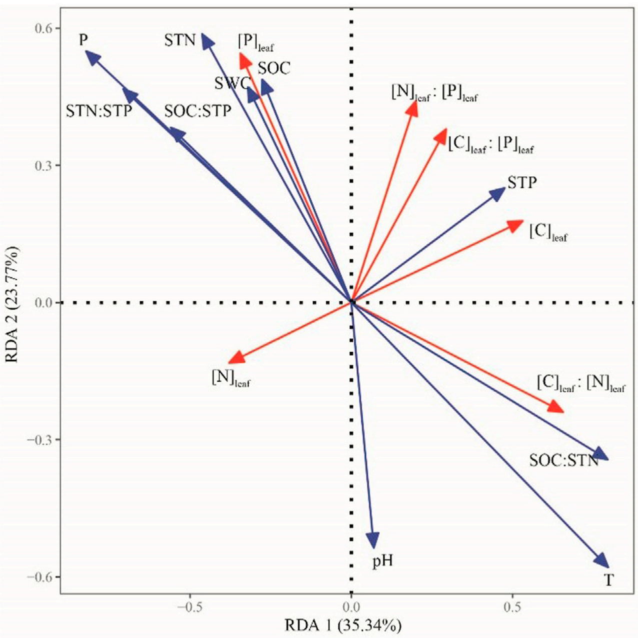


**FIGURE 3 |** Redundancy analysis (RDA) ordination for the leaf stoichiometric traits of *L. virgaurea* and environmental characteristics. pH, soil pH; SWC, soil water content; SOC, soil organic carbon concentration; STN, soil total nitrogen concentration; STP, soil total phosphorus concentration; $[C]_{leaf}$, leaf carbon concentration; $[N]_{leaf}$, leaf nitrogen concentration; $[P]_{leaf}$, leaf phosphorus concentration; T, temperature; P, precipitation.

metals in the soil increased, forming insoluble substances (e.g., Fe-phosphorus, Al-phosphorus, etc.), leading to a reduction in the availability of soil nutrients and making it difficult for plants to take up nutrients from the soil (Buchkowski et al., 2015). At this point, the plants may promote their own reabsorption of nutrients in order to maintain the nutrients for growth, resulting in the increase of $[N]_{leaf}$ (Aerts, 1996).

## Mechanisms for the Dominant Factors Affecting $[P]_{leaf}$ Across Elevations

The precipitation indirectly affected $[P]_{leaf}$ of *L. virgaurea* through three paths, namely STP-STN: STP path, SWC-STN: STP path and SWC-STP- STN: STP (**Figure 4C**). These paths can be explained: precipitation can influence soil nutrient concentrations and uptake effectiveness, along with soil water content (Liu et al., 2019a). For instance, with an increase in precipitation, the P leaching process is accelerated, resulting in a decreased STP concentration (Jiang et al., 2019). Increased precipitation can also lead to a high SWC, and reduce microbial activity (Borken and Matzner, 2009; Jiang et al., 2019). As microbial activity decreased, the conversion and use of STP by microbes would be reduced or stopped, resulting in increasing STP and consequently reducing the STN: STP ratio (Taylor et al., 1989). Temperature only indirectly affected $[P]_{leaf}$ of *L. virgaurea* through one path (**Figure 4C**). The reason for this is that low temperature can reduce microbial activity (Borken and Matzner, 2009; Jiang et al., 2019) and increase STN: STP as mentioned above.

With STN: STP influenced by temperature, precipitation and SWC, it exerted a direct effect on $[P]_{leaf}$ of *L. virgaurea*. Generally, STN: STP affects plant productivity and composition, as well as the composition and biological activity of soil microorganisms (Mulder and Elser, 2009; Zhao et al., 2018; Liu et al., 2010). With a change in the effective soil P concentrations that can be absorbed and utilized by plants due to biological activity influenced by environmental changes (Ouyang and Norton, 2019), $[P]_{leaf}$ will be affected. However, SEM showed that environmental factors can explain only about 21% of the variation of $[P]_{leaf}$ in the study area (**Figure 4**), suggesting that other factors such as species' own genetic characteristics may be more important for determining $[P]_{leaf}$ of *L. virgaurea*; further study is needed.

## Implications for Grassland Management

Since the leaf ecological stoichiometry of *L. virgaurea* was mainly affected by climate (temperature and precipitation) (**Table 3**), future climate change is expected to have an impact on the growth of *L. virgaurea* in the study area. The $[C]_{leaf}$: $[N]_{leaf}$ and $[C]_{leaf}$: $[P]_{leaf}$ not only reflect plants' N and P utilization efficiency (Reich et al., 2006; Sun et al., 2017a), but is also indicative of the rate of plant growth (Jiang and Song, 2010) — with lower $[C]_{leaf}$: $[N]_{leaf}$ and $[C]_{leaf}$: $[P]_{leaf}$ values, a faster growth rate is achieved (Jiang and Song, 2010; Yan et al., 2015). The temperature and precipitation will both increase across the QTP and thus at the study site. The increase in temperature in high elevation areas will be more significant than at lower elevations. The magnitude of change in precipitation is low in comparison with that of temperature (Liu et al., 2009; Hu et al., 2015; Wang et al., 2019). Based on this, we can infer that with future temperature increases, leaf nutrient concentration compensating metabolic rates will decline in high elevation areas, while the catabolic reaction processes of the soil-microbe-mediated soil nitrogen cycle will increase, as will nitrogen mineralization (Dai et al., 2020). As a result, more nutrients could be assimilated and allocated to vegetative

**TABLE 3 |** Level of explanatory capacity and significance of environmental factors in the variation of *L. virgaurea* leaf ecological stoichiometry at different elevations.

| Environmental factors | Explains (%) | F | P |
|---|---|---|---|
| SWC (%) | 15.6 | 11.5 | **0.008** |
| pH | 0.3 | 0.2 | 0.750 |
| SOC ($g{\cdot}kg^{-1}$) | 1.0 | 0.7 | 0.440 |
| STN ($g{\cdot}kg^{-1}$) | 0.2 | 0.1 | 0.870 |
| STP ($g{\cdot}kg^{-1}$) | <0.1 | <0.1 | 0.984 |
| SOC: STN | 0.7 | 0.5 | 0.512 |
| SOC: STP | 2.8 | 2.2 | 0.116 |
| STN: STP | 1.3 | 1.0 | 0.330 |
| T (°C) | 21.9 | 11.2 | **0.002** |
| P (mm) | 31.3 | 11.4 | **0.002** |

*pH: soil pH; SWC: soil water content; SOC: soil organic carbon concentration; STN: soil total nitrogen concentration; STP: soil total phosphorus concentration; T: temperature; P: precipitation. Significant values are in bold.*

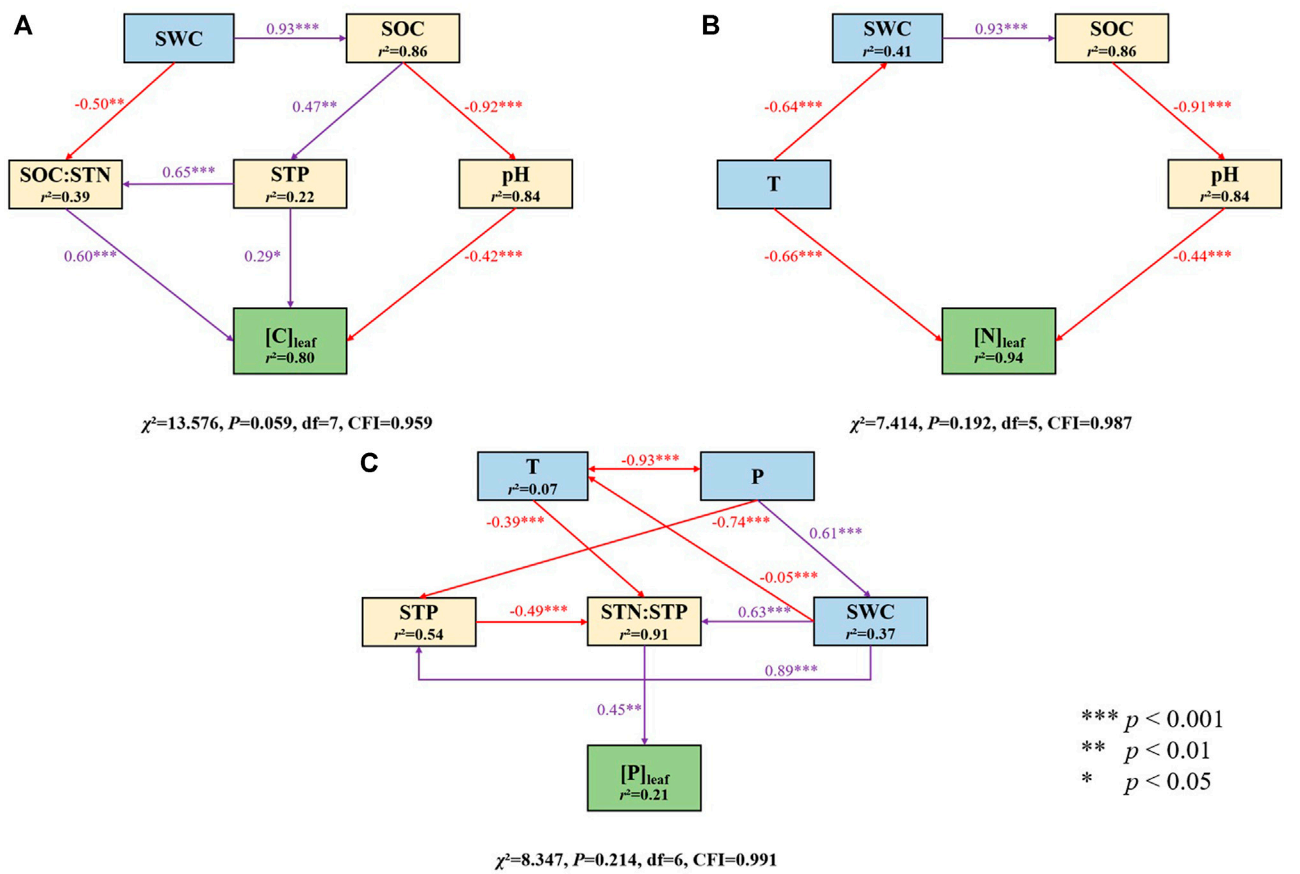


**FIGURE 4 |** Structural equation model (SEM) showing the direct and indirect effects of SWC, temperature and precipitation on $[C]_{leaf}$ **(A)**, $[N]_{leaf}$ **(B)** and $[P]_{leaf}$ **(C)**. pH, soil pH; SWC, soil water content; SOC, soil organic carbon concentration; STN, soil total nitrogen concentration; STP, soil total phosphorus concentration; $[C]_{leaf}$, leaf carbon concentration; $[N]_{leaf}$, leaf nitrogen concentration; $[P]_{leaf}$, leaf phosphorus concentration; T, temperature; P, precipitation.

growth, thereby promoting the growth of *L. virgaurea*. However, as $[N]_{leaf}$: $[P]_{leaf}$ of *L. virgaurea* in the study area exceeded 16 (mean = 17.93), *L. virgaurea*'s growth will be restricted by P (**Figure 2F**) (Koerselman and Meuleman, 1996; Güsewell, 2010). Nevertheless, determining plant nutrient restriction status based solely on $[N]_{leaf}$: $[P]_{leaf}$ does not tell the full story and environmental factors should be introduced as auxiliary evaluation criteria to further study the impact of nutrient restriction on *L. virgaurea* (Güsewell, 2010; Batjes, 2014; Niu et al., 2021).

For alpine grazing meadows, previous studies have found that the invasion of *L. virgaurea* can increase above- and below- biomass, SOC, STN and microbial biomass C and N concentrations in the topsoil (Shi et al., 2011; Shi et al., 2018). This means that the type of grassland degradation caused by *L. virgaurea* is harmful to plant diversity, especially to grasslands' high quality forage diversity, and eventually to livestock grazing, but it is not harmful to soil C and other soil nutrients. However, a decline in plant species will affect the stability of grassland ecosystems and decrease other services such as biodiversity protection (Báez et al., 2008; Das et al., 2019). This suggests that there must be a trade-off between soil function and supporting biodiversity threatened by the future spread of *L. virgaurea*. At present, both are very important for the QTP and all over the world, and thus the spread of *L. virgaurea* must be limited in scope.

## CONCLUSION

Based on field sampling in the northeast QTP and subsequent experimental analysis, *L. virgaurea* $[N]_{leaf}$: $[P]_{leaf}$ showed no significant changes with elevation; nevertheless, $[C]_{leaf}$, $[N]_{leaf}$, $[P]_{leaf}$ and $[C]_{leaf}$: $[N]_{leaf}$, $[C]_{leaf}$: $[P]_{leaf}$ fluctuated with rising elevation, confirming the fact that *L. virgaurea* can adjust its internal physiological characteristics or adopt different resource utilization strategies to adapt to environmental changes. Based on $[N]_{leaf}$: $[P]_{leaf}$, we found *L. virgaurea*'s growth to be susceptible to P limitation. However, such putative nutrient limitations must be further studied through a combination of leaf ecological stoichiometry, actual soil nutrient levels and the genuine nutrient requirements of plants in the study area.

The SWC, temperature and precipitation were the main environmental factors modulating leaf ecological stoichiometry of *L. virgaurea* across elevations. The SWC indirectly affected $[C]_{leaf}$ and $[N]_{leaf}$, temperature directly affected $[N]_{leaf}$, while $[P]_{leaf}$ was affected by all their indirect effects. As the contribution of temperature and precipitation to the total variance of leaf ecological stoichiometry was greater than that of SWC, climate change would have a dominant impact on the growth of *L. virgaurea*. Based on scientific predictions of future climate by researchers and combined with our research results, future climate change would promote the growth of *L. virgaurea*. Consequently, limiting the spread of *L. virgaurea* may require biological or chemical control in future grassland management.

The environmental factors investigated here only partly explain the changes in leaf ecological stoichiometry of *L. virgaurea* with elevation, which may also be related to other factors, such as vegetation community structure at different elevations, species richness, soil microbial diversity, etc. Moreover, only three elevations were studied here, over limited gradients. Consequently, to further understand the adaptation strategy of *L. virgaurea* in the context of grassland degradation, it is necessary to increase the elevation gradient, and explore the changes in ecological stoichiometry in *L. virgaurea* at different elevations.

## DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

## AUTHOR CONTRIBUTIONS

JJC contributed to the conception and planning of the research, and critically reviewed the manuscript, as did JA and AB. XZ and HS performed the experiments and analyzed the data. JBC and HS checked the format and wrote the article. All authors have read and agreed on the final contents of the manuscript. The authors read and approved the final manuscript.

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